Chapter 5
Induced Pluripotent Stem Cells and Vascular Disease

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What Are iPS Cells?

Induced pluripotent stem cells (iPS cells) are adult cells which have been reprogrammed to an embryonic-like state by forced over-expression of genetic factors important in the maintenance of Embryonic Stem Cells (ESCs). They are similar to ESCs in both morphology and phenotype, expressing stem cell markers and having the ability to generate all three germ layers [1]. iPS cells are very useful multi-purpose tools offering the potential for exciting possibilities in the field of regenerative medicine.

Role of iPS Cells in Regenerative Medicine

Pluripotent stem cells like iPS cells could be customised to be patient-specific, avoiding, thus, problems arising due to tissue rejection. It would also circumvent the need for immunosuppressive drugs and their adverse side-effects in patients.

A particularly appealing aspect of using iPS cells is that these cells can be directed to differentiate into any cell lineage, paving the way for treatment of many types of diseases. The potential medical applications are numerous and range from treating many diseases, such as Alzheimer's or Parkinson's disease, cardiovascular disease and diabetes to cellular tissue regeneration [2, 3].

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In fact, iPS cell technology has already revolutionised the fields of regenerative medicine; for example, it has successfully treated sickle-cell anemia in a mouse model [4] and also provided scientists with powerful laboratory models for studying the manifestation of particular diseases. These include hepatic [5], neurological [6], endothelial [7] and cardiovascular [7].

In addition, iPS cells can be useful tools in drug development and also assist researchers in intervening and correcting the genetic defect at its root, before the onset of the disease.

Advantages and Disadvantages of iPS Cells

Advantages

iPS cells are remarkable research tools. They are similar to ESCs and can serve as models towards the understanding of the complex series of events during embryonic development or a certain disease by allowing researchers the detailed study of their mechanisms. One of the biggest advantages with using these cells is the avoidance of immune rejection, as they can be derived from a patient's own cells. Using iPS cells also avoids the ethical issues linked to the use of human embryos in medical research.

Disadvantages

One of the main disadvantages in using iPS cells in cellular reprogramming and regenerative medicine is the fact that the process is generally slow and with low levels of efficiency [8]. The other major disadvantage is the tumourigenic potential of iPS cells [9]. Indeed, several in vitro studies have shown that the reprogramming process has the ability to produce genetic and epigenetic changes in iPS cells [10, 11]. Another problem with using iPS cells in the study of disease models stems from the fact that iPS cell lines are highly heterogeneous leading to intrinsic variability, and, thus, different observed phenotypes. It is, therefore, important to evaluate several cell lines from both the same patient and different patients.

iPS Cells Generation

One of the most common methods of iPS cell generation is transduction of the reprogramming genes to the cells of interest using integrating retroviral or lentiviral vectors. The most widely accepted method of iPS cell generation involves the genetic transduction of a combination of reprogramming factors, namely Oct4, Sox2, Klf4 and c-Myc, (OSKM) using retroviral or lentiviral vectors (Fig. 5.1). It was first discovered by Takahashi and Yamanaka after screening of pre-selected

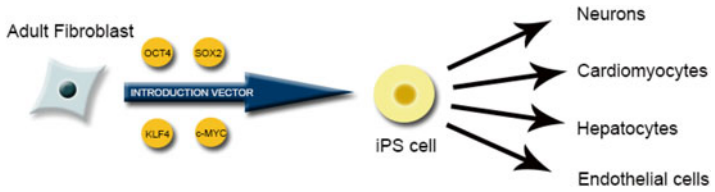

Fig. 5.1 Classic example of iPS cell generation using the OCT4, SOX2, KLF4, and c-MYC transcription factors through an introduction vector, and subsequent differentiation to the desired cell lineage

factors in mouse embryonic fibroblasts (MEFs) [12]. This combination has been shown to work in other somatic cell types and different species too, including monkey [13] and human [14].

Later studies used different combinations of reprogramming factors such as Oct4, Sox2, Nanog and Lin28 [15] while more recent studies have used even fewer factors; in neural stem cells, expression of only one factor (Oct4) was shown to be sufficient to induce pluripotency [16].

The above approaches, however, present an obstacle towards the clinical translation of iPS cells due to their potential tumorigenicity. Studies have tried to address this problem using either minimal genetic modifications [17] or the use of non-integrating vectors [18]. Non-integrating vectors, either viral or non-viral, that have been used successfully to generate iPS cells include adenoviruses, the Sendai virus, expression plasmids, minicircle vectors, and liposomal magnetofection [19].

Protein transduction of OSKM is another promising alternative approach [20]. In addition, reprogramming of iPS cells and differentiation to the desired cell type has also been made possible with the use of microRNAs (miRNAs), which are small non-coding RNAs that can regulate gene transcription [21, 22].

The chemical approach of using small molecules to enhance reprogramming efficiencies or even replace certain reprogramming factors is also among the methods that may offer an alternative solution. Some DNA methyltransferase inhibitors, histone deacetylase inhibitors (valproic acid) which modulate chromatin modifications have been reported to enhance the reprogramming process [23–26].

Other methods involve the generation of iPS cells using episomal vectors from a variety of cells such as fibroblasts or bone marrow mononuclear cells. They are introduced into the system by electroporation, providing a transgene-free, virus-free iPS cell generation [27].

Regenerative Medicine and Vascular Disease

Regenerative medicine methodologies that aim to recover cardiac and vascular function are being increasingly explored as management approaches for vascular and cardiovascular diseases. However, one of the biggest obstacles towards applying such therapeutic approaches is the reduced availability of suitable cells needed for clinical

purposes. For example, in the field of cardiac disease, which is a leading cause of mortality and morbidity worldwide [28, 29], a very large number of healthy cells would be required for use in a clinical setting. Since cell regeneration is quite limited in the adult heart [30], urgent development of fast and robust new therapies that will produce clinical-grade cells, suitable for disease modelling, tissue engineering, and cell replacement treatments is imperative. Endothelial cells (ECs) play a very crucial role during the development of vascular and cardiovascular disease. Although just a thin cellular monolayer lining the inner walls of blood and lymphatic vessels, the healthy endothelium is implicated in a wide range of factors. It is central to cardiovascular homeostasis [31], as well as in regulating vascular tone and, thus, blood pressure. It also plays a crucial role in cell adhesion, fluid filtration, smooth muscle cell proliferation and vessel wall inflammation. ECs also play an important role acting as barrier between a vessel's lumen and surrounding tissues, as well as in blood clotting (thrombosis/fibrinolysis) and repair of damaged vascular cells [32, 33]. It is, thus, important to note their key role while researching potential solutions in vascular diseases.

What Is Vascular Disease?

Vascular disease is an abnormality of the blood vessels and involves a narrowing of a vessel's diameter leading to obstruction of normal blood flow. It is caused by atherosclerosis, which can block arteries in critical parts of the body. Atherosclerosis is a disease, which causes plaque build-up in the inner walls of an artery (Fig. 5.2).

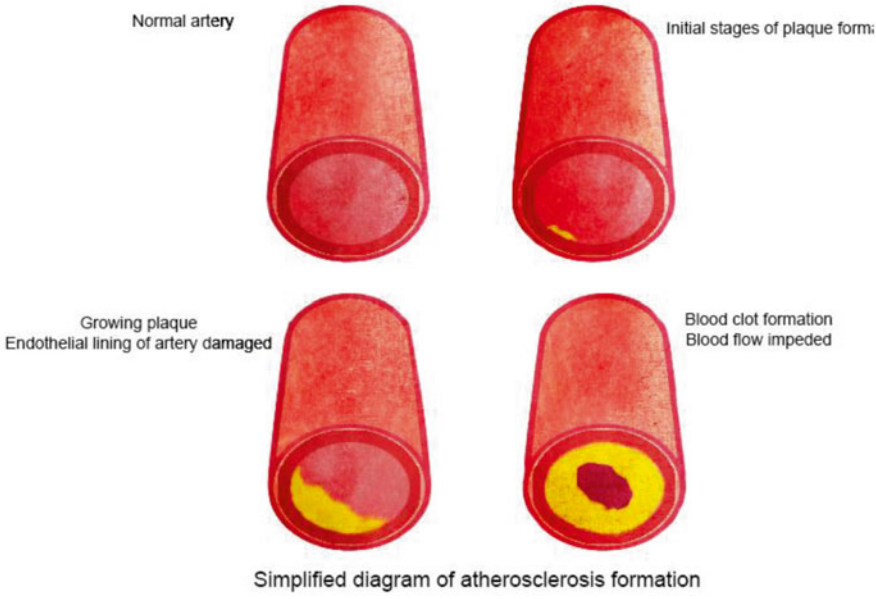

Fig. 5.2 Simplified diagram of atherosclerosis formation

Plaque is a mixture of fat deposits, cholesterol, calcium and other cellular debris and it can lead to serious problems that include heat attack, stroke and, on certain occasions, even death. It is particularly dangerous as, many times, there are no symptoms preluding the onset of a critical episode.

It is now commonly accepted that at the heart of vascular disease lies endothelial cell dysfunction. The vascular endothelium is not just a simple barrier between intravascular and interstitial compartments, it is also responsible for the regulation of hemodynamics, the angiogenic remodelling of vessels, as well as a plethora of metabolic, anti-inflammatory, and antithrombogenic processes [34].

Types of Vascular Diseases

There are various types of diseases involving the blood vessels. These include the following:

Peripheral Arterial Disease

Peripheral arterial disease (PAD), also called peripheral vascular disease (PVD) or peripheral artery occlusive disease (PAOD), is a condition in which the fatty deposits build up (plaque) in the outer arteries of the body (arms, legs) cause a narrowing of the artery wall, decreasing blood flow and supply.

Symptoms: The symptoms of PAD include pain, numbness fatigue, and muscle discomfort in the lower limbs. They may appear slowly but can increase in frequency over time. In severe cases, PAD symptoms may lead to night cramps, feet and toes tingling, dark and blue skin appearance (cyanosis), non-healing sores and hair loss in the affected area.

Risk Factors: The disease most commonly affects men over 50 years of age but it can also affect women. Smoking is the main risk factor, while other factors such as age, abnormal cholesterol, increased blood pressure and having a history of certain diseases such as diabetes, heart and kidney or cerebrovascular disease (strokes) also increase the risk. PAD also increases the risk of coronary heart disease, strokes, and heart attacks.

Detection: In an examination, indicative findings of PAD may include decreased blood pressure or weak/absent pulse in the affected limb and calf muscle atrophy. Blood tests may show diabetes or high cholesterol. Other tests include arteriography on the legs, ankle/brachial index (ABI), Doppler ultrasound and Magnetic resonance angiography (MRA).

Treatment and Management: Giving up smoking and balancing exercise with rest can help alleviate the symptoms by improving blood circulation. Weight loss in cases of obese patients and lowering of cholesterol may also prove beneficial. Keeping blood sugar under control is also essential in cases of diabetes. Medicines

prescribed by the doctor for controlling the disorder include aspirin, and other anti-coagulants such as clopidogrel for preventing blood clot formation. Artery-dilating drugs such as cilostazol may also be prescribed in more severe cases when surgery is not an option. Anti-cholesterol drugs may also be prescribed. As a last option, surgery may also be performed and may involve artery angioplasty and stent placement or peripheral artery bypass.

Outlook: Very rarely the limb may need to be amputated, especially in cases involving gangrene development. In most cases, however, PAD can be sufficiently controlled without the need for surgery [35].

Aneurysm

An aneurysm is a swelling that resembles a balloon-like structure in a vessel. It can grow large and eventually rupture or dissect. If any of these occur, the outcome is usually fatal. Aortic aneurysmal disease was previously believed to be a form of atherosclerosis, but is now recognised as degenerative process involving all layers in a vessel wall. Its pathophysiology mainly involves four events: lymphocytes and macrophages infiltration of the vessel wall; collagen and elastin degradation in the media and adventitia; loss of smooth muscle cells; and neovascularization [34].

There are three main types of aneurysms: aortic, cerebral and peripheral aneurysms.

Aortic aneurysm is sub-classified into thoracic aortic aneurysm (TAA) and abdominal aortic aneurysm (AAA):

TAA occurs in the aorta running through the chest (Thorax), in which the arterial walls close to the heart weaken leading to improper heart valve closure and subsequent blood leakage back into the heart.

AAA occurs in the aorta that runs through the abdominal area and is located between the diaphragm and the aortic bifurcation. It is a full-thickness dilatation on a part of the vessel that exceeds the normal vessel diameter by 50 %. Typically though, an aneurysm diameter of 3.0 cm is usually regarded as the threshold. When identified, these aneurysms are typically monitored for expansion. The growth rate can vary depending on the individual. It is usually characterised by progressive expansion, with some remaining stable for years, while others may grow rapidly. The most common predictor of AAA rapture is the aneurysm's size. Most of them are asymptomatic until they rapture and they can be often lethal. Therefore, the main goal is to be able to identify them and treat them before the point of rapture. Aneurysms are classified as suprarenal if they involve at least one visceral artery, pararenal if they involve the origins of renal arteries, and infrarenal if they begin beyond the renal arteries. Key risk factors for AAA include ageing, male gender, and family history. In men aged 50 or over and women 60–70 or over, the incidence of AAA increases significantly with each passing decade [34]. Other risk factors for AAA include smoking, hypertension, increased cholesterol, obesity and atherosclerotic occlusive disease [34]. Aneurysms are commonly discovered during routine abdominal examinations. However, ultrasonography is the principal method of screening with a very high

sensitivity and specificity [34]. Treatment usually involves risk factor modification such as smoking cessation or control of co-existing conditions that contribute to the risk with the use of medication (for example, statins or antihypertensive agents).

Cerebral or intracranial aneurysm is a cerebrovascular disorder which occurs in an artery of the brain. If rapture occurs, blood leaks into the area around the brain (subarachnoid haemorrhage). Aneurysms are classified as saccular, fusiform and microaneurysms.

Saccular (berry) aneurysms are the most common and appear as a round outpouching. They are almost always the result of an inherited blood vessel weakness and usually occur within the arteries of the Circle of Willis. Fusiform ones usually appear in an arterial segment around the entire vessel rather than just one side of the vessel wall. Microaneurysms (or Charcot-Bouchard aneurysms) occur in small blood vessels. The vessels most commonly affected in this type of aneurysm are the lenticulostriate vessels in the basal ganglia. Small aneurysms are relatively symptomless but if they rapture, they may cause an intracerebral haemorrhage. Larger aneurysms also produce no symptoms, but on occasions a person may experience sudden and severe headaches, nausea, sight impairment and unconsciousness prior to the rapture. If rapture occurs, blood leaks into the area around the brain (subarachnoid haemorrhage). Risk factors include lifestyle-originating diseases such as smoking, excess alcohol consumption, obesity and hypertension. Trauma to the head or infections may also contribute to the development of an aneurysm. Genetic conditions have also been linked to increased risk. They include autosomal dominant polycystic kidney disease, neurofibromatosis type I, Marfan syndrome, pseudoxanthoma elasticum, hereditary hemorrhagic telangiectasia, Ehlers–Danlos syndrome type II and IV and multiple endocrine neoplasia type I [36]. Once suspected, brain aneurysms can be diagnosed with medical tests such as angiography, magnetic resonance imaging and CT scans. Emergency treatment after rapture generally involves improving respiration and reducing intracranial pressure. This is achieved through surgical clipping or endovascular coiling [37, 38].

Peripheral aneurysms occur in areas other than the chest and brain. They most commonly develop in the popliteal artery in the lower part of the thigh and knee but they can also occur in the femoral and carotid arteries or arteries in the arm. As with other types of aneurysms, peripheral ones have common risk factors such as obesity, smoking, high cholesterol and high blood pressure, as well as family history of heart disease. Some of the symptoms include a throbbing lump in the affected limb, claudication (cramping), numbness and pain. Diagnostic tools include CT scans, MRI and ultrasound while treatment may require thrombolytic therapy or surgical repair [39].

Renal Failure

Renal failure (kidney failure or renal insufficiency) is a medical condition that affects the function of the kidneys, which receive their blood supply from the aorta through the renal arteries. Kidneys are particularly sensitive to any decrease in blood flow, and, thus, a narrowing of the renal arteries due to plaque build-up, can

lead to serious complications. One of the main functions of the kidneys is eliminating waste products generated as a result of the body's metabolism, extracting them from the blood and sending them to the bladder through the ureter. Urea is one of the major waste products. In renal failure occurring as a result of vascular disease, the kidneys fail to adequately filter these waste products. Renal failure, which has five stages (number 5 being the most severe), is determined by the decrease in glomerular filtration rate, the rate of blood filtration in the renal glomeruli. It is usually detected by a decrease or non-passage of urine or accumulation of waste products, like creatinine or urea, in the blood [40].

There are two types: acute kidney injury, which is usually reversible and chronic kidney disease, which is usually not reversible and there may be an underlying cause. In acute failure, there is a rapid loss of renal function, which is accompanied by oliguria (decreased urine production) as well as an electrolyte imbalance. It can be the result of a number of causes, which are generally classified as prerenal, intrinsic and postrenal. Chronic renal disease may have numerous causes, the most common being diabetes mellitus and long-term hypertension. Overuse of common drugs such as aspirin and paracetamol may also lead to chronic renal disease [41].

Renal disease symptoms may include nausea and vomiting, weight loss, blood in the urine (uremia), and changes in the frequency of urination (more or less frequent) due to the high urea levels in the blood. Other symptoms caused by build-up of inadequately filtered phosphates in the blood may include bone damage and muscle cramps [42]. Build-up of potassium blood levels may lead to hyperkalaemia and abnormal heart rhythm or muscle [43] paralysis. Other symptoms include pain, swelling, polycystic kidney disease or anaemia with resulting fatigue and dizziness.

Treatment options for renal failure mainly involve dialysis to remove waste products and excess fluid from the blood. Transplantation is also another option; however sometimes health issues may prevent taking this route.

Diabetic Vascular Disease

Diabetic Vascular Disease refers to artery blockages throughout the body because of diabetes. In diabetes, blood sugar levels are elevated due to the body's inability to either produce insulin or to use it effectively. The majority of patients with diabetes exhibit abnormalities of endothelial function and vascular regulation. The factors involved in diabetic endothelial dysfunction are numerous but a key final common pathway is the deregulation of nitric oxide (NO) bioavailability. NO is a key stimulus for vasodilation and also inhibits vascular smooth muscle proliferation migration or proliferation. It also limits activation of platelets. Hyperglycemia inhibits the function of eNOS in endothelial cells and increases reactive oxygen species (ROS) production. In addition, insulin resistance may also contribute to loss of normal NO homeostasis [44]. The sum effect of the deregulation of these mechanisms and of endothelial cell dysfunction increases the inflammatory state of the vessel wall.

This process is accompanied by increased leukocyte chemotaxis, adhesion and transformation into foam cells, which is an early precursor of atheroma formation [45]. Apart from changes in pathways involving endothelial cells, diabetes also stimulates pro-atherogenic mechanisms in vascular smooth muscle cells in a similar fashion [46].

Symptoms include blurred vision, limb swelling, foot sores, pain and high blood pressure. Initial assessment in patients with diabetes begins with a thorough medical history and examination [47]. Other than the standard glucose tests, the physician, depending on the affected organ, may order tests to determine and monitor the function of, for instance, blood vessels, eyes and kidneys. The final diagnosis and treatment will usually require the collaboration of physicians from different fields.

The Need for Novel Therapies

Everyone is at risk of developing vascular disease, with millions of people around the world suffering from adverse complications related to it, which are, in many cases, lethal. Vascular disease ranges from diseases affecting the arteries, veins and lymphatic vessels to disorders that affect blood circulation causing ischemia.

Vascular disease is one of the leading causes of death in the western world and results from the monolayer of cells lining the vessels, endothelial cells, becoming dysfunctional. This results in the downstream effects of disease such as atherosclerosis. The repair and regeneration of these cells has therefore been the focus of research for many years however to date still faces many barriers. In recent years, there has been great advancement in the generation of iPS cells and their ability to differentiate towards a specific lineage. In terms of vascular disease, the research is aimed towards the generation of functional vascular cells with the goal of regeneration of the vascular tissue as well as personalised medicine via the use of autologous tissue. However, the underlying mechanisms and signalling pathways that are involved in the differentiation process to produce optimal endothelial cells are generally unknown.

Potential for iPS Cells to Differentiate Towards Vascular Cells

Recent ability to derive vascular cells through reprogramming from iPS cells holds huge therapeutic potential for personalised medicine and vascular cell therapy. Stem cells are intricately coupled with their extracellular surroundings; therefore any range of extrinsic signals that causes change to their environment have a direct effect on their subsequent response, such as remaining in the same state or inducing differentiation towards a specific cell within the three germ layers. For example, cells can remain in a pluripotent state by being cultured in conditions that block reprogramming such as leukaemia inhibitory factor (LIF) [48].

Similarly, adding a dynamic array of factors and signals that mimic the elements seen during organogenesis in development can induce the pluripotent cell to differentiate into the desired specific cell line [49, 50].

One of the many advantages of using iPS cells is the exciting idea of personalised medicine through the use of autologous tissue. For example, the generation of vascular cells from the patient's own cells overcomes the limitations, such as tissue rejection [51], seen in embryonic stem cells.

Cardiovascular disease is one of the leading causes of death in the western world and current therapy is limited. The generation of vascular cells from iPS cells offers a new window for this research that will overcome these limitations. The ability to generate vascular cells from iPS cells allows close study and better understanding of the generally unknown underlying mechanisms in vascular differentiation. Elucidating these mechanisms will result in the generation of efficient protocols for the development of functional vascular cells for therapy.

Mechanisms Involved in iPS-Derived Differentiated Vascular Cells

It is widely known and practiced that vascular cells can be generated from iPS cells; however the underlying mechanisms involved are poorly understood. Signalling pathways involved in vasculogenesis/angiogenesis (Fig. 5.3) and defects in vascular remodelling are seen in pathways such as Notch, Wnt, VEGF, TGFβ [52–56] and mutations in these pathways respectively. These pathways work independently and also simultaneously with each other. Vasculogenesis occurs almost exclusively during embryogenesis as it is the generation of vessels with no pre-existing vessel. The mesodermal differentiates into hemangioblasts which aggregate and form blood islands consisting of endothelial precursor cells (EPCs) and hematopoietic stem cells (HSCs).

Notch Signalling

These blood islands fuse and become primitive capillary plexus which sends signals to recruit more cells and also for progenitor mural cells to differentiate in order to remodel and develop a mature blood vessel [52, 53, 57] (Fig. 5.3). Angiogenesis will occur after a stimulus such as tissue wounds, inflammation or pathogenic responses such as vascular supply to tumors. These stimuli create a hypoxic environment which in turn results in the production of growth factors such as vascular endothelial growth factor (VEGF). This causes the basement membrane to become disrupted and upregulation of a member of the NOTCH pathway,

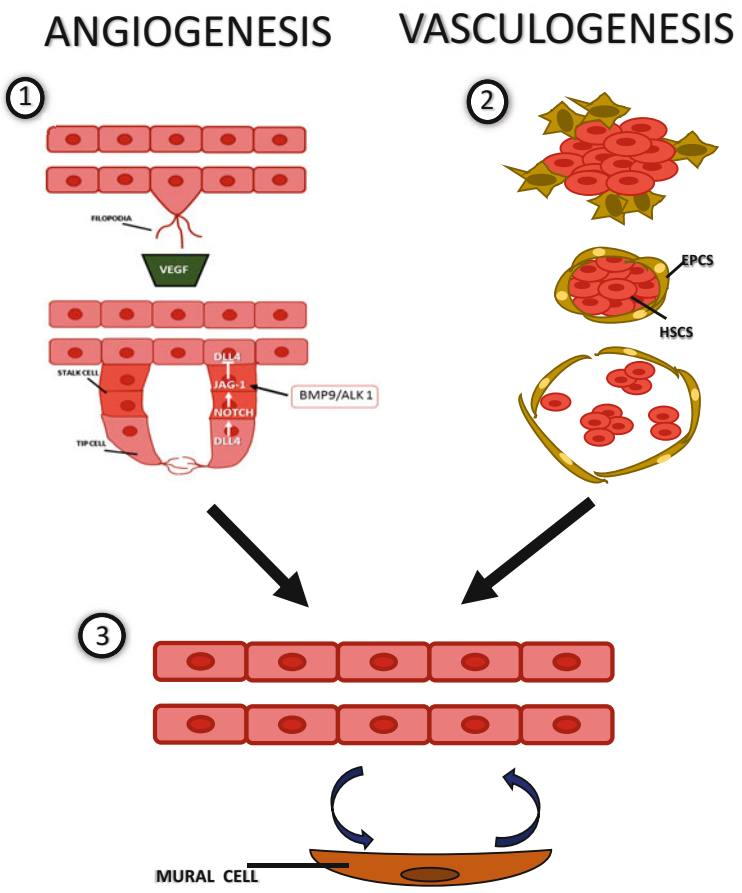

Fig. 5.3 Diagram showing the stages of angiogenesis [1] and vasculogenesis [2] and the recruitment of mural cells to nascent vessel [3]

delta like ligand—1 (DLL-4) in ECs and causing it to adapt the morphology of a "tip cell." VEGF receptor A (VEGFRA) becomes upregulated and drives the tip cell towards the VEGF stimulus using membrane extensions called filopodia. However, these tip cells do not divide, it is the preceding cells known as "stalk cells" that proliferate and form the new vessel wall. In these stalk cells, DLL4 upregulates NOTCH signalling which aids in proliferation (working alongside Wnt signalling). At the same time, DLL4 upregulates NOTCH receptor jagged-1 which inhibits activation of DLL4 in adjacent cells therefore stopping these cells becoming tip cells which regulates and controls angiogenesis.

MicroRNA 199b

Other factors can also influence the outcome of these pathways, for example, Micro RNA 199b has been shown to modulate vascular cell fate through targeting and suppressing the expression of Jag-1 which then in turn activates STAT3 expression which binds to the promoter of VEGF and results in induction of EC differentiation [58].

TGFB Signalling

TGFβ signalling can work as pro- or anti-angiogenic in many ways. Here Bone morphogenic protein 9 (BMP9) and activin-like receptor-like kinase 1 (ALK1), members of the TGFβ pathway are seen here to help regulate angiogenesis by regulating jagged-1 expression [52, 53, 59, 60] (Fig. 5.4). TGFβ/BMP work in a sensitive dose-dependant manner which regulate their effect on angiogenesis (Fig. 5.4). TGFβ binds to TGFBR2 recruiting TGFBR1 (ALK-5) and ACVRL1 (ALK-1). TGFB is pro-angiogenic at low doses (parallel to a high concentration of BMP9) causing high binding to ALK-1 receptor and the downstream effects of this results in EC proliferation and migration needed for angiogenesis to occur. The opposite dose of these factors causes this to be inhibited through binding of ALK-5 and the associated downstream responses [52, 59, 60]. Micro RNA 27 has been shown to

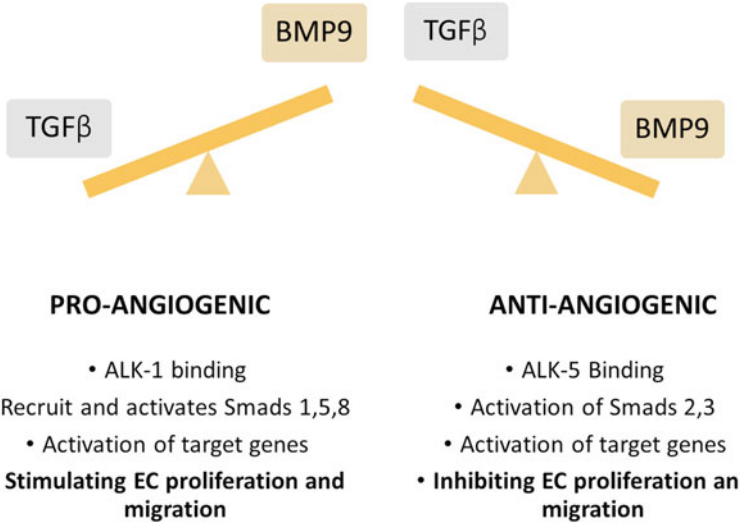

Fig. 5.4 Diagram illustrating how TGFB family affects angiogenesis in a dose-dependant manner

effect the expression of EC markers as when it is overexpressed there is an increase in the EC marker presence and this is due to an increase in the TGFBR2 [61].

Eventually sprouting tip cells will anastamose forming a new vessel. These ECs require support and structure from mural cells (pericytes and vascular smooth muscle cells, vSMCs) which will also help the vessel with vasoconstriction and dilation. ECs secret platelet-derived growth factor (PDGF) recruits the mural cells and in a reciprocal signalling mechanism, these mural cells secret VEGF [52, 53, 62–65]. Contrastingly, mural cells will then maintain vessel stability through Angiotensin—Tie2 signalling [59, 60].

Wnt Signalling

VEGF, NOTCH and TGFβ signalling are some of the most prevalent signalling pathways involved in vascular cell development and maintenance. There are however many other signalling pathways that interact and also work independently to obtain the same outcome. Wnt signalling is involved in many cellular processes such as proliferation and maintenance of stem cells in the undifferentiated state. Canonical Wnt signalling has been shown to regulate VEGFA expression through β-catenin expression [52, 66, 67]. β-catenin expression has been seen to increase during proliferating vessels stimulating the VEGFA promoter and therefore angiogenesis [67]. Tight regulation of β-catenin is therefore required to regulate angiogenesis. Histone deacetylase 7 (HDAC7) has been shown to interact with β-catenin reducing the expression and therefore keeping ECs in a low proliferative state [68].

Studying these pathways in detail allows us to elucidate the key factors necessary for differentiating successfully and efficiently iPS cells to functional vascular cells for future developments in therapy.

RNA Binding Proteins

There are also many correlating genes in vascular generation that can be studied in detail to understand the mechanisms. For example, the quaking gene (QKI) has been shown to have a major role in vascular development. QKI belongs to the family of highly conserved RNA binding proteins called STAR (Signal Transduction and Activation of RNA). It is a pre-transcription regulator, meaning it controls aspects such as pre-mRNA splicing, mRNA stability and protein translation [7, 56, 69–73]. QKI was originally associated and defined for its involvement in myelinisation and oligodendrocyte differentiation [7, 70, 74–76]; however, more recently it has been discovered for its involvement in vascular development [7, 56, 71, 72, 76] prior to the start of myelination, this is seen clearly in vivo where qki null mice were embryonic lethal between E9.5 and E10.5 due to a failure of blood circulation in the yolk sac [7, 56, 69, 72].

QKI has been shown to have a key involvement in embryonic blood vessel formation and remodelling. It is the preliminary defects in the provascular endothelium that cause the lethal vascular defects. During normal vasculogenesis development the endoderm and mesoderm interact producing signals causing the cells to differentiate to endothelium and some erythrocytes which form blood islands. These blood islands fuse and become primitive capillary plexus which sends signals to recruit more cells and also for progenitor mural cells to differentiate in order to remodel and develop a mature blood vessel. QKI is expressed in the endoderm layer regulating its function and when this is not present it causes these series of vascular differentiation events to become dysfunctional. The cells are unable to differentiate to mature vascular smooth muscle cells and it is this perturbed investment of mural cell to the nascent vessels that causes the yolk sac vasculature to become unstable and inhibit the essential remodelling required for progression of development resulting in embryonic death [7, 69, 71].

Chromatin Remodelling Mediators

A gene defined as "similar to SET translocation protein" (SETSIP) has been discovered to be expressed in parallel with endothelial markers via microarray analysis. The SET protein is involved in essential cell processes such as chromatin remodelling, differentiation [77], apoptosis and cell cycle progression [78]. Several transcript variants encoding different isoforms have been found for this gene. SET protein is part of a complex localised to the endoplasmic reticulum but is also found in the nucleus [79]. Indeed, depletion of SET by RNA interference (RNAi) delays transcription, suggesting a positive role in transcription [80]. Overexpression of SETSIP resulted in a correlating increase in EC markers and contrastingly a decrease in expression when it is knocked out; therefore there is a strong connection with SETSIP and the regulation of endothelial differentiation from pluripotent cells. In particular, luciferase assays have shown that SETSIP translocates to the nucleus and binds to the promoter of the endothelial structural marker VE-cadherin, which is an essential molecule in maintaining EC structure and integrity. Further studies also showed that SETSIP expression was induced by VEGF [81].

At the moment, the potential of the iPS cells to differentiate towards therapeutic cells is only based on directed empiricism, while they are totally dependent on combinations of growth factors, media, and matrices to favour the desired lineage. In regards to vascular regeneration, it is important to understand the key regulatory pathways such as epigenetic alterations, transcriptional activity and RNA binding patterns associated with the differentiation processes. Only then, fully defined experimental protocols could reproducibly guide iPS cells to a vascular lineage [82, 83] and enable clinical application [84–86].

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